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Sensors and Actuators B 106 (2005) 394–400
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

A hydrogen peroxide biosensor based on nano-Au/PAMAM dendrimer/cystamine modified gold electrode

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Received 30 March 2004; received in revised form 7 August 2004; accepted 24 August 2004
Available online 25 September 2004

Abstract

Horseradish peroxidase (HRP) to a nano-Au monolayer, which was supported by PAMAM dendrimer/cystamine modified gold electrode. The high affinity of PAMAM dendrimer for nano-Au with its amine groups was used to realize the role of nano-Au as an intermediator to retain the high bioactivity of HRP. A hydrogen peroxide biosensor was fabricated based on the effective immobilization of the high bioactivity of HRP. In the presence of the hydroquinone mediator in the solution, the immobilized HRP exhibited excellent electrocatalytical response to the reduction of H_2O_2. The effects of various experimental variables were optimized. The resulting biosensor showed a linear response to H_2O_2 over a concentration range from 1×10^{-5} to 2.5×10^{-3} mol L^{-1} with a sensitivity of 0.53 A L mol^{-1} cm^{-2} and a detection limit of 2.0 μmol L^{-1} based on a signal-to-noise ratio of 3. The apparent Michaelis-Menten constant (K_m^{app}) of the biosensor was evaluated to be 0.52 mmol L^{-1}. The biosensor exhibited high sensitivity and good stability and reproducibility. Performance of the biosensor was evaluated with respect to possible interferences and the application to real sample analysis.

Keywords: Nano-Au; PAMAM dendrimer; Horseradish peroxidase; Biosensor

1. Introduction

Hydrogen peroxide is an essential mediator in food, pharmaceutical, clinical, industrial and environmental analyses [1,2], so the determination of hydrogen peroxide is practically important. Numerous techniques such as titrimetry [3], spectrometry [4], and electrochemistry [5–10] have been employed for this purpose. Among the electrochemical techniques, an amperometric biosensor based on electron transfer between an electrode and immobilized horseradish peroxidase (HRP) is especially attractive because of its simplicity and high sensitivity [6–9].

The self-assembly technique was often used as a method for the fabrication of amperometric biosensors [11–13]. The biosensors with self-assembly technique can dramatically reduce non-Faradaic background currents resulting in improved sensitivity [14]. In self-assembly process, gold is the commonly used electrode material. Deng and co-workers studied a reagentless H_2O_2 biosensor based on thionine covalently tethered to multilayer HRP in a self-assembly monolayer as an electron-transfer mediator [15]. However, the covalent linking between the enzymes and the tailed groups immobilized on the electrode surface may decrease the bioactivity of enzymes. In recent years, nano-scaled particulate gold (nano-Au) has been extensively studied in analytical chemistry for its special physico-chemical characteristics. Colloidal gold sols as a base for the immobilization of enzymes without loss of their bioactivity have been reported [16,17]. Also, Chen and co-workers have used self-assembly technology to immobilize HRP on Au colloid nanoparticles for the construction of HRP biosensor [18,19].

Dendrimers belong to a new class of synthetic macromolecules characterized by a regularly branched treelike structure. Also, dendrimers with high generation numbers

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doi:10.1016/j.snb.2004.08.023

($G \geq 4$) usually possess a nearly spherical shape [20]. There have been increasing interests in applying dendrimers to the related areas such as drug delivery, energy harvesting, ion sensing, catalysis, and information storage [21]. Among various dendrimers, poly(amidoamine) (PAMAM) dendrimers are the most frequently studied. In recent years, some reports have shown that the PAMAM dendrimers can be used as the bioconjugating reagents for construction of glucose oxidase electrode [22–24]. A HRP biosensor utilizing the adsorption of chitosan to nano-Au was prepared in this laboratory [25]. The chitosan monolayer on the electrode has a planar structure, and the amount of adsorbed nano-Au is limited which affects the amount of HRP immobilized. In the present work, we have focused on the study of the suitability of the fourth-generation (G4) PAMAM dendrimers for the development of the H_2O_2 biosensor using self-assembly technique. First, the G4 PAMAM dendrimers were covalently linked to cystamine-modified Au electrode through glutaraldehyde cross-linking. With the help of the amino groups located on its surface, the nano-Au particles are adsorbed. Finally, HRP was attached to the electrode surface via nano-Au. When G4 PAMAM dendrimers were immobilized on the electrode, owing to their spherical structure, the surface area and the density of amino groups on the electrode increased, the amount of adsorbed nano-Au on the electrode was also increased, this would result in the increase of immobilized HRP. Thus, a higher sensitivity could be expected as compared with that of the conventional biosensors. The determination of H_2O_2 was performed with the aid of the electron mediator hydroquinone. In the presence of HRP, H_2O_2 oxidized mediator$_{(Red)}$ into mediator$_{(Ox)}$, the later was subsequently reduced at the electrode with an applied potential of -200 mV versus saturated calomel electrode (SCE). The optimized conditions for the fabrication and analytical performance of the enzyme electrode were studied. The stability and reproducibility of the electrode were also evaluated.

2. Experimental

2.1. Materials

Horseradish peroxidase (HRP, E.C.1.11.1.7, $A > 250\,U\,mg^{-1}$) and cystamine were purchased from Sigma. The glutaraldehyde (25% water solution) was obtained from Shanghai Biochemical Reagents (Shanghai, China). H_2O_2 (30% w/v solution) was from Beijing Chemical Reagents (Beijing, China), and the concentration of dilute H_2O_2 prepared from this material was determined by titration with potassium permanganate. $AuCl_3HCl{\cdot}4H_2O$ (Au > 48%) and all other chemicals were of analytical grade and used without further purification. All solutions were made up with doubly distilled water.

The supporting electrolyte was $0.05\,mol\,L^{-1}$ phosphate buffer solution (PB), prepared with KH_2PO_4 and Na_2HPO_4.

2.2. Apparatus

Cyclic voltammetric and amperometric experiments were performed using a PAR 273 potentiostat/galvanostat and model 270 software (EG & GPrinceton Applied Research, Princeton, NJ, USA). A conventional three-electrode system was employed with the H_2O_2 sensor as working electrode, a Pt foil as auxiliary electrode, and a saturated calomel electrode (SCE) as the reference against which all potentials were measured. Experiments were carried out in an electrochemical cell holding 10 mL of $0.05\,mol\,L^{-1}$ PB (pH 7.0). Cyclic voltammetric measurements were done in an unstirred solution. Amperometric experiments were performed in a stirred solution with a steady-state background current first obtained before aliquots of H_2O_2 standard solution were successively added to the cell. All experiments were carried out at room temperature.

2.3. Preparation of amine-terminated G4 dendrimers

According to the method reported by literature [26], ethylene diamine, served as a core, was treated with methyl acrylate at 40–60 °C in methanolic solution, produced half generation ester terminated dendron. Subsequent reaction of this half generation dendrimer with excess of ethylene diamine gave the first generation amine-terminated dendrimers (G1). Repetition of these operations mentioned above yielded the larger amine-terminated dendrimers (G4).

2.4. Preparation of Au colloids

All glassware used in the following procedure were cleaned in freshly prepared aqua regia (3:1 HNO_3–HCl) and rinsed in doubly distilled water. Au colloids were prepared according to the literature [25] by adding sodium citrate solution to a boiling $HAuCl_4$ aqueous solution. Preparations were stored in dark glass bottles at 4 °C. The diameter of the Au colloid was 20 ± 2.1 nm, which was measured by using transmission electron microscopy (TEM).

2.5. Construction of the H_2O_2 sensor

Fig. 1 describes the basic strategy for the preparation of H_2O_2 biosensor. A gold electrode was constructed by sealing polycrystalline gold wires (99.99%, 0.3 mm in diameter) in a soft glass tube. Before each new measurements, the gold electrode was polished with 1.0, 0.5, and 0.3 μm alumina slurry, sonicated consequentially in distilled water and absolute ethanol for 15 min each and etched finally in $0.5\,mol\,L^{-1}$ H_2SO_4 solution by cyclic-potential scanning between -0.3 and + 0.8 V until a reproducible voltammetric response was obtained.

The cleaned gold electrode was first immersed in $20\,mmol\,L^{-1}$ cystamine aqueous solution for 2 h. After the electrode was thoroughly rinsed with water to remove physically adsorbed cystamine, it was transferred into 5% (v/v)